

DNA Extraction Using Liquid Nitrogen in *Staphylococcus aureus*

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Nowadays, polymerase chain reaction (PCR) is among the fastest diagnostic way of diseases, in which desoxy nucleic acids (DNA) is an inseparable part for this purpose. There are different approaches to extract the DNA, which are all supported by using the expensive chemical enzymes, however due to lack of access to most of the enzymes and high cost, it is also possible to use substitution cost effective, faster methods, with much more extraction as yield at the end of the procedure (Bergdoll, 1990; Stephan, et al., 2001; Pourgholam et al., 2011).

Staphylococcus aureus was considered as the objective species for testing a substitutional method in PCR. Generally, the enzymes used to lyse the cell wall of the *Staphylococcus aureus* in molecular diagnosis are: motilyzine, lysostafine, lysozyme and etc. In the present study, various approaches such as sonication, freeze-thawing and the liquid

nitrogen were used to get cell lysate. The best result was obtained by using liquid nitrogen with -196°C. *S. aureus* (RTCC 2411) ¹ samples were stored at -20°C and kept until bacteriological test.

Before molecular analysis, the specimens were aseptically transferred to culture medium for microbial analysis. Samples were streaked onto brain heart infusion broth (Merck), mixed and incubated overnight at 37°C. After incubation, the growing colonies were picked up and used for bacterial DNA isolation using the method described by Gillespie and Oliver (2005). Identification of the isolate was done by cultural, morphological and biochemical characters according to Da Silva, et al. (2004, 2005). Results were compared to determine the sensitivity of the multiplex PCR assay in identifying bacteria with culture.

Growth colonies sample was transferred into 1.5 ml micro tube and

bacterial cells were precipitated by centrifugation at $3,000\times g$ for 5 min at 4°C , and aqueous phase discarded. The amount of $30\ \mu\text{L}$ of SDS 10 percent was added to the bacterial cells and transferred to sterile mortar that contains liquid nitrogen (LN) with enough volume. After freezing, the cells were squeezed by formation of crystals. Bacterial cell wall was smashed with pestle mechanical strikes and disrupted without using any lytic enzymes based on the method described before to extract the fungal DNA. After transferring the lysed cells to the

micro tube, Phenol-chloroform was added and centrifuged in $10,000\times g$ for 10 min. Supernatant (phenol) was removed and chloroform and alcohol (1:24) was added and centrifuged at $10,000\times g$ for 2 min and $20\ \mu\text{l}$ RNase was added. The amount of $7.5\ \mu\text{l}$ NaCl for precipitation of DNA and $600\ \mu\text{l}$ isopropanol were added and kept in room temperature (RT) for 10 min. After centrifugation at $10,000\times g$ for 10 min at 4°C , supernatant was removed and the precipitate, which contains DNA was washed two times with 70% of ethanol and dissolved in distilled H_2O .



Figure 1: DNA bands on lane 1, 2, 3 and 4; M, DNA ladder (Cinnagen); lane 5 is blank.

The result of the DNA extraction by using liquid nitrogen is demonstrated in Figure 1, which obtained in 1.5 percent agarose gel. This method is very suitable for gram-positive bacteria, specially *staphylococcus spp*, with stiff wall due to the peptidoglycan. The lasted time to extract

DNA from bacteria was roughly estimated in 15 min. By using liquid nitrogen not only the application of expensive commercial enzymes were omitted, which is very cost effective, but also the spent time decreased to a point which is equal to the half of the time comparing with the

commercial enzymes. The method of liquid nitrogen described in our method is completely competitive with the other classical methods. For example, in sonication method, where the outcome is not satisfied and the noise of the apparatus is intolerable; in glass bead method in which the DNA is chopped and the obtained results is not satisfying (Fader et al., 1987; Jorgensen, H. J., 2005).

According to the previous methods such as freeze-thawing which is time consuming to repeatedly freeze and heat the bacterial samples the obtained results is not satisfying. In the present study, liquid nitrogen was employed without using any expensive and specific enzymes necessary to each bacterium; we hopefully wish to observe our new method to be tested by the other researchers and to be substituted with the methods which are currently used.

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